CLAIMS

1. A modified bacterial surface layer (S-layer) protein, the modification comprising the internal insertion of a heterologous polypeptide.

2. The protein according to claim 1 wherein:

(a) the unmodified protein is from a Gram positive or non-aquatic bacteria, optionally a lactic acid bacteria or *Lactobacillus*; and/or

(b) the heterologous polypeptide is a functional polypeptide or a polypeptide of interest, optionally a binding or targeting protein (such as an antigen, antibody, or part thereof).

3. A protein according to claim 1 or 2 wherein:

(c) the protein retains most of the full length sequence of the unmodified S-layer protein;

(d) the polypeptide is inserted at an internal location at least five amino acids from the C or N terminus; and/or

(e) the modified protein has a size of from 40 to 70 kDa.

4. A protein according to any preceding claim which:

(f) has a crystallisation or C-terminal domain that is predominantly basic, or hydrophobic,

(g) an N-terminal domain which is either predominantly hydrophilic; or

(h) has alternating hydrophobic and hydrophilic regions.

5. A protein according to any preceding claim wherein the heterologous polypeptide is inserted at a location in the protein either so that it is :

(i) exposed, or present on the cell surface;

(j) present in the surface layer, or the cell wall;

(k) is protected from external proteolytic processing or is not recognised or bound by external antibodies.

6. A protein according to any preceding claim wherein the modified or unmodified protein:

(l) crystallises, optionally into an oblique lattice (such as of p1 or p2 symmetry);

(m) has a cell wall anchor domain;

(n) has a pI of at least 7; and/or

(o) is predominantly basic.

7. A protein according to any preceding claim wherein the polypeptide comprises an antigen causing or specific for a disease, and optionally is an antigen recognisable by an antibody or is all or part of an antigen from an anaerobic bacteria, optionally *Clostridium*.

8. A protein according to any preceding claim which (in unmodified form) is from *Lactobacillus acidophilus*, *crispatus*, *helveticus*, *amylovorus*, or *gallinarum*.

9. A fragment of a bacterial surface layer (S-layer) protein which is:

a) an N-terminal fragment or a fragment that is capable of forming a dimer with an other such fragment or a trimer with two other such fragments;

b) capable of forming dimers with another such fragment and either

(i) includes an immunodominant or exposed loop region and is from 20 to 200 amino acids long; or

(ii) excludes an entire immunodominat or exposed loop region and is from 20 to 155 amino acids long.

10. A polynucleotide encoding a protein according to any of claims 1 to 9.

11. A vector comprising a polynucleotide according to claim 10.

12. A host cell comprising, or which is has been transformed with a vector according to claim 11.

13. A bacteria expressing a surface layer protein (or fragment) according to any of claims 1 to 9.

14. A bacteria according to claim 13 which is a lactic acid bacteria, optionally from *Lactobacillus*, and is preferably *L. plantarum*, *L. acidophilus* or *L. casei*.

15. A modified bacteria (other than *L. casei* or *Bacillus*) which has been modified to express a heterologous surface layer (S-layer) protein.

16. A bacteria according to claim 15 which would not normally, or as a wild-type or in unmodified form does not, possess a surface layer.

17. A modified bacteria according to claim 15 or 16 which is a *Lactobacillus* cell and/or the S-layer has its own, original, cell wall anchor.

18. A bacteria according to any of claims 15 to 17 which is a *Lactobacillus* bacterial cell, such as *L. casei*, and/or the S-layer protein is from *Lactobacillus* bacteria, such as *L. acidophilus*.

19. An *L. casei* bacterial cell expressing a bacterial surface layer (S-layer) protein that is either not from *L. crispatus* or is not a collagen bidning protein.

20. An *L. casei* cell according to claim 19 wherein the S-layer protein is, or is derived, from *L. acidophilus*.

21. A modified bacteria expressing only, or homogeneously, a heterologous or modified surface layer (S-layer) protein.

22. A bacteria according to claim 21 having a genome which includes a polynucleotide encoding a heterologous S-layer protein, optionally integrated into the genome, and/or where the polynucleotide encoding the normal or wild-type S-layer protein has been silenced, replaced, switched off or otherwise rendered non-expressed.

23. A bacteria according to claim 22 or 23 wherein the heterologous or modified S-layer protein is the sole or only S-layer protein expressed by the bacterial cell and/or the cell does not express any wild-type S-layer protein.

24. A bacteria according to any of claims 22 to 24 wherein the S-layer protein is located on the surface of the cell wall and/or a multiplicity of S-layer proteins form an S-layer.

25. A vaccine comprising a bacteria according to any of claims 13 to 24.

26. A vaccine according to claim 25 which is an oral or nasal vaccine and/or additionally comprises an adjuvant.

27. A sheet or (optionally crystalline) monolayer or 2-dimensional array comprising a plurality of bacterial surface layer proteins, at least one of which is modified protein according to any of claims 1 to 8.

28. A solid surface, liquid-air interface, lipid film, liposome or solution comprising a sheet, monolayer or array according to claim 27.

29. A solid surface according to claim 28 to which is bound one or more (macro) molecules, such as an enzyme, antibody or other binding molecule, receptor, antigen or ligand.

30. A solid surface comprising a layer of S-proteins, at least a plurality of which are modified proteins according to any one of claims 1 to 8, sandwiched between the surface and a layer of functional molecules.

31. A sensor, molecular sieve or ion trap comprising a sheet, layer or array according to claim 27 or a surface according to any of claims 28 to 30.